Descriptions of first-instar nymphs of the mealybugs *Antonina tesquorum* Danzig and *Nesticoccus sinensis* Tang (Hemiptera: Coccoidea: Pseudococcidae) with a key to known species in the genus *Antonina*

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Abstract: The microscopic morphologies of first-instar nymphs of *Antonina tesquorum* Danzig and *Nesticoccus sinensis* Tang are described and illustrated in detail for the first time. The relationship between *A. tesquorum* and *A. graminis* is discussed. A key to known species of the genus *Antonina* in the world is provided based on first-instar nymph morphology.

Key words: immature stage; morphology; taxonomy

远东安粉蚧和竹巢粉蚧的一龄若虫形态记述及世界安粉蚧属一龄若虫分种检索表（半翅目：蚧总科：粉蚧科）

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摘要：首次对远东安粉蚧 *Antonina tesquorum* Danzig 和竹巢粉蚧 *Nesticoccus sinensis* Tang 一龄若虫的显微形态进行详细描述，并提供了相关特征图。讨论了远东安粉蚧与九龙安粉蚧 *A. graminis* 之间的关系，并编制了世界安粉蚧属已知一龄若虫分种检索表。

关键词：幼期；形态；分类

Introduction

Pseudococcidae is the second largest family of Coccoidea (Tang 1992) with approximately 2018 species in 270 genera worldwide (Ben-Dov *et al*. 2014). At present, the classification in the family Pseudococcidae relies primarily on the morphological characteristics of the adult female (Williams 2004) and only 89 species are described at the immature stages (Ben-Dov *et al*. 2014). Some morphological characters such as the legs and antennae are useful in the identification of mealybugs; however these characters are missing or reduced in the adult females of some groups such as *Antonina* (Yang & Kosztarab 1967). Therefore the classification based on adult females in these groups must depend on other morphological characters and has hindered the identification of these species (McKenzie 1960). However, in all species, the first-instar nymphs have developed legs and antennae. The

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①Corresponding author, E-mail: sananwu@ bjfu.edu.cn taxonomy based on immature stages has proven helpful in determining relationships within Diaspididae, another family in the superfamily Coccoidea (Howell & Tippins 1990). Therefore, there is a need to adequately describe and illustrate the first-instar nymphs of more species in the Pseudococcidae.

The genus *Antonina* is one of the oldest genera in the family Pseudococcidae (Signoret 1875) and now includes 29 species (Ben-Dov *et al.* 2014). In eleven species, the first-instar nymphs have been described: *Antonina australis* Froggatt, *A. graminis* (Maskell), *A. maai* Williams & Miller, *A. milleri* Williams, *A. nakaharai* Williams & Miller, *A. nanlingensis* Wu & Lu, *A. natalensis* Brain, *A. pretiosa* Ferris, *A. purpurea* Signoret, *A. transvaalensis* Brain and *A. zonata* Green (Yang & Kosztarab 1967; Williams & Miller 2002; Williams 2004; Wu *et al.* 2012). The genus *Nesticoccus* is regarded as closely related to the genus *Antonina* (Wu 2001). *Nesticoccus* includes 2 species and *N. sinensis* Tang is its type species (Tang 1992; Ben-Dov *et al.* 2014). The first-instar nymph of *N. sinensis* has been described by Xie & Yan (1983) and Tang (1992), but both descriptions are incomplete.

The aims of this paper are: 1) to describe the first-instar nymph of *A. tesquorum* for the first time; 2) to redescribe the first-instar nymph of *N. sinensis* Tang in detail, and provide a modern illustration; 3) to provide a key to species of genus *Antonina* based on first-instar nymphs for known species; and 4) to discuss the relationship between *A. tesquorum* and *A. graminis*.

Material and methods

The specimens examined, including adult females and first-instar nymphs, were collected by the authors, preserved in 75% alcohol, and deposited in the Insect Collection, the Department of Forestry Protection, Beijing Forestry University, Beijing, China (BFUC). Slide-mounted specimens were prepared using the method of Borchsenius (1950), stained in acid fuchsin and mounted with Canada balsam. The specimens of adult females were used for species identification and those of first-instar nymphs for description and illustration.

Morphological examination was made with an Olympus BX40 compound microscope and a Leica DME compound microscope. Drawings were outlined using a Leica drawing tube. Details and enlargements were drawn using the 100X objective with oil immersion. Morphological terms follow those of Yang & Kosztarab (1967) and Williams (2004). Measurements were made under a phase-contrast microscope (Olympus BX40) fitted with an ocular micrometer and were based on the number of specimens specified in the description of the first-instar. Measurements are in micrometers (μm) except that length and width of body are in millimeters (mm) and are typically represented by the mean followed by the range in parentheses. Each figure has a central drawing for the whole specimen, the left half representing the dorsal surface and the right half representing the ventral surface. Enlarged drawings show the structure of important features.

Taxonomy

Key to species of the genus *Antonina* based on the morphology of first-instar nymphs for known species (modified from Wu *et al.* 2012)

1. Trilocular pores of one size ··2
-. Trilocular pores of two or three sizes···5
2. Anterior ostioles present··3
-. Anterior ostioles absent··4
3. Circulus present between abdominal segments III and IV; dorsal trilocular pores not forming distinct longitudinal rows··*A. zonata* Green
-. Circulus absent; dorsal trilocular pores forming 8 distinct longitudinal rows on abdominal segments············ ··*A. australis* Green
4. Dorsal trilocular pores forming 2 distinct longitudinal rows on abdominal segments·············· *A. panica* Hall
-. Dorsal trilocular pores forming 6 distinct longitudinal rows on abdominal segments···*A. transvaalensis* Brain
5. Circulus present··6
-. Circulus absent··9
6. Dorsal trilocular pores forming 4 distinct longitudinal rows on abdominal segments································ 7
-. Dorsal trilocular pores not forming distinct longitudinal rows·· 8
7. Dorsum usually with eight setae present on each abdominal segment·····························*A. milleri* Williams
-. Dorsum usually with six setae present on each abdominal segment··············*A. nakaharai* Williams & Miller
8. Line of small-sized trilocular pores around body margin; trilocular pore associated with each ostiole··········· ··*A. maai* Williams & Miller
-. No line of small-sized trilocular pores around body margin; no trilocular pore associated with ostioles········· ··*A. pretiosa* Ferris
9. Anterior ostioles present; anterior spiracle associated with 3 or 4 trilocular pores····*A. nanlingensis* Wu & Lu
-. Anterior ostioles absent; anterior spiracle associated with 1 or 2 trilocular pores······································10
10. Anterior spiracle associated with 1 trilocular pore; ventrally the anal lobe without a bar seta······················ ··*A. purpurea* Signoret
-. Anterior spiracle associated with 2 trilocular pores; ventrally the anal lobe with a bar seta······················ 11
11. Dorsal trilocular pores forming 4 distinct longitudinal rows on abdominal segments; ventral abdominal segments II–VII, each with 1 simple disc pore on each side of body margin············· *A. graminis* (Maskell)
-. Dorsal trilocular pores forming 6 distinct longitudinal rows on abdominal segments; only 2 simple disc pores singly on submargin of prothorax ·· *A. tesquorum* Danzig

1. First-instar nymph of *Antonina tesquorum* Danzig (Fig. 1)

Mounted material. Body elongate-oval, 0.51−0.55 (0.53) mm long, 0.23−0.25 (0.24) mm wide. Antennae 6-segmented, 132−143 (137.8) µm long, apical segment longest, nearly 2/5 total length of antennae; length of segments as follows (µm): I 19−22 (20.8), II 15−19 (17.3), III 16−17 (16.5), IV 13−16 (15.3), V 18−21 (19.3), VI 48−50 (48.8). Basal segment trapezoidal, with 3 hair-like setae and 1 spine; segment II bowl-like, with 3 hair-like setae and 1 sensorium apically; segment III cylindrical with 4 hair-like setae, segment IV also cylindrical with 5 hair-like setae; segment V narrow ring-like, with 5 hair-like setae and 1 fleshy setae; apical segment elongate ovoid, with 18 hair-like setae and 4 fleshy setae. Eyes round, present on margin of head. Mouthparts well developed, clypeolabral shield rectangular in shape, about 1.5 times longer than wide, 100−110 (104.3) µm long and 68−73 (71) µm wide, with 2 setae. Labium widely triangular, with 3 segments, but segmentation indistinct, approximately 1/2 length of clypeolabral shield, 43−50 (46.5) µm long, 47−57 (50.8) µm wide, with 11 pairs of labial setae as follows: 7 pairs on apical segment, 1 pair on medial segment, 3 pairs on basal segment. Stylet coiled, extending to fifth abdominal segment. Legs well-developed, 3 pairs, trochanter triangular, each with 7 setae and 2 sensoria on each face, distal trochanteral setae slender, each about 45 µm long, longer than other trochanteral setae; femur enlarged; tarsus with 1 sensorium at its base and a pair of tarsal digitules arising from its apex, tarsal digitules equal in size, both slightly expanded at apex; claw stout, without denticle, with a pair of disparate claw digitules, one normal and knobbed, the other shorter, more slender and only slightly knobbed, both longer than claw. Length of hind legs (µm): coxa 25−28 (26.3), trochanter + femur 88−95 (90.5), tibia + tarsus 104−106 (104.8), claw 11−14 (12.5). Ratio of lengths of hind trochanter +femur to tibia+tarsus 0.84−0.90 (0.86) : 1, ratio of lengths of hind tibia to tarsus 1.06−1.17 (1.10) : 1. Spiracles in 2 pairs, small trumpet-shaped. Anterior spiracular arm 17−19 (18) µm long, including peritreme, atrium 9−11 (9.9) µm wide; posterior spiracular arm 19−21 (19.8) µm long, atrium 9.5−12 (10.4) µm wide. Each spiracle associated with 2 trilocular pores, 3.5−4.5 (3.9) µm in diameter. Circulus apparently absent. Anal lobes slightly protruding, each ventral surface with 1 apical seta, each 172−198 (182) µm long, with basal boss 6.5−7.5 (7) µm wide; 1 subapical setae present near each apical seta, each 30−40 (33.8) µm long, with basal boss 3.5−5.0 (4.3) µm wide; anal lobe bar absent. Anal ring situated at apex of abdomen, more or less circular, 38−42 (40.3) µm in diameter, with 2 rows of irregularly shaped pores, pores of marginal row smaller than those of inner row. Three pairs of anal ring hairs, length of each pair: anterior 43−45 (43.8) µm; median 41−60 (49.3) µm; posterior 34−42 (38.3) µm. Ostioles with inner edges of lips not sclerotised. Only posterior pair present, between sixth and seventh abdominal segments, with 1 trilocular pore on each lip and 1 seta about 9.4 µm long on anterior lip. Cerarii on anal lobes only, each anal-lobe cerarius with two enlarged conical setae, 22−27 (24.3) µm long, usually with one trilocular pore between conical setae and no auxiliary setae. Simple disc pores much smaller than a trilocular pore, each 2.8−3.2 (3.0) µm in diameter, only 4 in number, singly on submargin of prothorax.

Dorsum. Dorsal surface with small sharply spinose setae, mainly short and slender on abdominal segments I–VI, thorax and head, each 6−11 (7.3) µm long, setae on abdominal segment VII longer, each 12−20 (16.8) µm long. There are 14−16 minute setae evenly scattered on head; 20 setae on prothorax and mesothorax; 6−14 setae scattered over metathorax; 8 setae in a transverse row on each of abdominal segments I–V, 4 in a transverse row on abdominal segment VI; 6 long setae on abdominal segment VII, and no setae on abdominal segment VIII in addition to those in cerarii. Trilocular pores of one type, each 4.6–4.8 (4.7) µm in diameter, evenly scattered on head and thorax, in transverse segmental rows on the abdomen, there are 4 on the head, 8−10 on prothorax, 14 on mesothorax, 4−6 on metathorax, 8 on abdominal segment I, 6 on each of abdominal segments II−IV, 8 on abdominal segment V, 4 on each of abdominal segments VI−VII except 4 associated with ostioles, and 6 on abdominal segment VIII: 4 arranged in a transverse row, the other two in cerarii. Vesicular protuberances present as a pair near base of antennae only, petal shaped, each 2.5−3.5 (2.9) µm in diameter.

Venter. Ventral setae lanceolate or flagellate, many of them longer than those on dorsum. 3 pairs of interantennal setae, each 37–40 (38.5) µm long; setae sparse on thorax, 5.5–20 (8.3) µm in length, 6 on prothorax; 6−8 on mesothorax; 6 on metathorax; setae on abdomen each 22−31 (27) μm long, gradually longer toward the posterior apex, abdominal segments II−VII, each with 6 in a transverse row, segment VIII with 2 enlarged setae on submargins. Two pairs distinct cisanal setae present on posterior abdominal venter: cisanal seta 21−24 (22.3) μm; obanal seta 18−23 (20.3) μm in length. Trilocular pores of two sizes; larger size each 4.8−5.5 (5.1) μm in diameter, present throughout the ventral surface, evenly scattered on head and thorax, in segmental rows on abdomen, usually a pore associated with each ventral seta; 2 on head; 14 on prothorax; 12 on mesothorax; 10 on metathorax; abdominal segments II−VI, each with 6 in a transverse row; segments VII−VIII, each with 4 in a transverse row. Smaller-sized triloculars each 3.5−4.5 (3.9) μm in diameter, present as a pair near each spiracular opening only.

Specimens examined. Eight first-instar nymphs, **China,** Jilin, Gongzhuling City, Xiangling Park, on leaf base and root of *Cleistogenes caespitosa*, 09-VIII-2013, Coll. San'an WU & Xubo WANG.

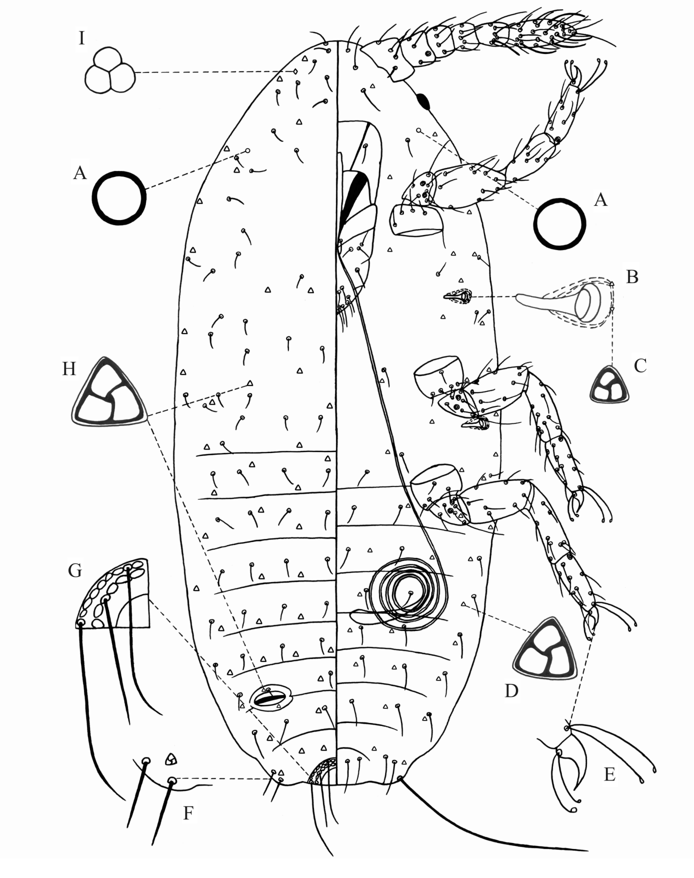

Figure 1. *Antonina tesquorum* (Danzig), first instar nymph. A. Simple disc pore; B. Thoracic spiracle; C. Ventral small type of trilocular pore; D. Ventral large type of trilocular pore; E. Claw; F. Anal lobe cerarius; G. Anal ring; H. Dorsal trilocular pore; I. Vesicular protuberance.

2. **First-instar nymph of *Nesticoccus sinensis* Tang** (Fig. 2)

Mounted material. Body elongate-oval, 0.51−0.56 (0.53) mm long, 0.27−0.29 (0.274) mm wide. Antennae 6-segmented, 128.5−130 (129.5) μm long, apical segment longest, nearly 1/3 total length of antennae; length of segments as follows (μm): I 21.5−23 (22.5), II 19−20 (19.4), III 14−15.5 (14.8), IV 14.5−16 (15.4), V 15−16 (15.6), VI 41−42.5 (41.9). Basal segment large and trapezoidal, with 3 hair-like setae and 1 spine; segment II cylindrical or trapezoidal, with 2 hair-like setae, 1 spine and 1 sensorium apically; segment III cylindrical with 4 hair-like setae, segment IV also cylindrical with 5 hair-like setae; segment V bowl-like, with 5 hair-like setae and 1 fleshy setae; apical segment elongate ovoid, with 18 hair-like setae and 4 fleshy setae. Eyes present, round and prominent. Mouthparts well-developed, clypeolabral shield more or less rectangular in shape, about 1.2 time longer than wide, 97−100 (98.5) μm long and 77−84 (80) μm wide with 2 setae. Labium 3-segmented, triangular, nearly 1/2 length and width of clypeolabral shield, 43−50 (46.5) μm long, 47−57 (50.8) μm wide, with 11 pairs of setae: apical segment with 7 pairs of setae, medial segment with 1 pair of setae, and basal segment with 3 pairs of setae. Stylet loop coiled, extending to end of fifth abdominal segment. Legs normal, robust, trochanter with 7 setae and 2 sensilla on each surface, distal trochanteral setae slender, each 33−35 (33.5) μm long, longer than other trochanteral setae; femur enlarged; tarsus with 1 sensorium at its base and a pair of tarsal digitules arising from its apex, tarsal digitules equal in size, both apically knobbed; claw thick and curved, with a small denticle and a pair of claw digitules, claw digitules subequal in length, but one much thinner than other, slightly expanded at apex, the other apparently knobbed, both slightly longer than claw. Length of hind legs (μm): coxa 25−30 (26.5), trochanter + femur 86−91 (89.1), tibia + tarsus 89−94.5 (91.8), claw 12.5−15.5 (14.1). Ratio of lengths of hind trochanter + femur to tibia + tarsus 0.94−1.02 (0.97) : 1, ratio of lengths of hind tibia to tarsus 0.92−1.05 (0.98) : 1. Length of fore legs and middle legs about same as hind legs. Spiracles small, trumpet-shaped. Anterior spiracular arm 15−19 (16.8) μm long, including peritreme, atrium 7−7.5 (7.3) μm wide, with 2 trilocular pores associated with it; posterior pair 18−20 (19) μm long, 6.5−8 (7) μm wide at atrium, only 1 trilocular pores associated with atrium. Circulus broadly elliptical, slightly notched laterally, situated between segments III and IV, 34–40 (36.3) μm wide, divided by intersegmental fold. Anal lobes slightly developed, 2 ventral setae at apices, each 62−84 (73.8) μm long, with basal boss 4.5−5 (4.6) μm wide; 1 subapical setae present near each apical seta, each 13−19 (16.8) μm long, with basal boss 2.2−3 (2.6) μm wide; anal lobe bar present. Anal ring oval in shape, situated at apex of abdomen, 32−36 (34) μm in diameter, with 2 rows of irregularly-shaped pores, and 3 pairs of anal-ring setae, length of each pair: anterior 19−26 (23) μm; median 14−17 (15.8) μm; posterior 18.5−21 (19.9) μm. Anterior pair of dorsal ostioles absent; posterior pair conspicuous, with inner edges of lips not sclerotized, with 1 trilocular pore on each lip and 1 seta about 9.8 μm long on anterior lip. Only anal lobe cerarii present, each with two conical setae and one trilocular pore, each setae 11−12.5 (12.1) μm long.

Dorsum. Dorsal setae present throughout the dorsum surface, each 7−18 (10.8) μm long, those on posterior abdominal segments longer than others. Head with 14 scattered setae; prothorax with 26; mesothorax with 18; metathorax with 8−9 in a transverse row. Setae are arranged in transverse segmental rows on abdomen, abdominal segments I−V each with 8 in a transverse row; segment VI, with 4 in addition to 2 on lip of ostioles; segment VII with 6;

setae absent from segment VIII except in cerarii. Trilocular pores of one type, each 3.2−3.6 (3.5) µm in diameter, mostly distributed on marginal and submarginal area. There are 4 on head; 6 on prothorax; 6 on mesothorax; 4 on metathorax; abdominal segments I−VII, each with 2 on each marginal area of the body; abdominal segments II−V, each with 2 in the submarginal area; segments VI−VII each with 2 in the median area of the body, and 2 on lips of the ostioles; segment VII with 6 pores: 4 in median area and 2 in cerarii. Vesicular protuberances present as a pair near base of antennae only, petal-shaped, each 2.5−3.2 (2.8) µm in diameter.

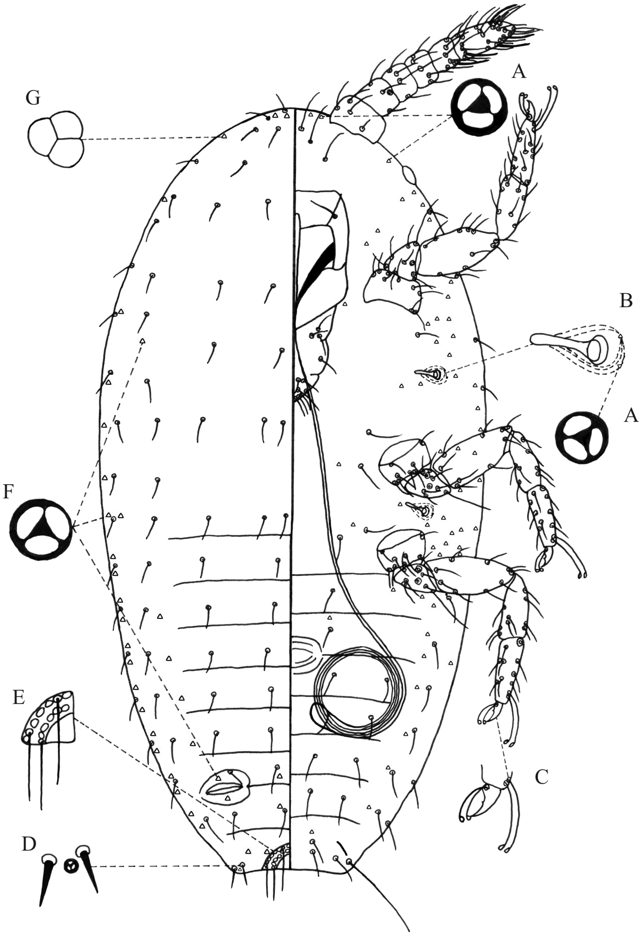

Figure 2. *Nesticoccus sinensis* Tang, first-instar nymph. A. Ventral trilocular pore; B. Thoracic spiracle; C. Claw; D. Anal lobe cerarius; E. Anal ring; F. Dorsal trilocular pore; G. Vesicular protuberance.

Venter. Ventral surface with normal flagellate setae present, mostly each 9–22 (16) µm long, usually longer than dorsal setae. Flagellate setae distributed as follows: with 3 pairs between antennae, each about 24–27 (25.8) µm long; sparse on thorax, 8 on prothorax; 6 on mesothorax; 2 laterad to each metathoracic leg; arranged in transverse segmental rows on abdomen, abdominal segments II−III each with 4; segments IV−V each with 6; abdominal segment VI−VII each with 8. Cisanal setae 13−15 (13.8) μm; obanal setae 12−13.5 (13) μm in length. Trilocular pores of two sizes, more numerous than in dorsum. A larger type each 3.2−3.5 (3.3) μm in diameter, occurring mainly around ventral margins, represented by 6 on margin of head; 32 on prothorax; 22 on mesothorax; 24 on metathorax; 8 scattered on abdominal segment II; abdominal segment III with 2 in each submarginal area, and 1 in each marginal area; abdominal segments IV−VII each with 1 on each side of the submargin; abdominal segment VIII with 2 in the median area. A smaller type each 3.0−3.1 (3.03) μm in diameter, present near the opening of spiracles only.

Specimens examined. Seven first-instar nymphs, **China,** Beijing, Zizhuyuan Park, under the leaf sheath of *Phyllostachys propinqua*, 10-VI-2014, Coll. Jing WAN, Xubo WANG & Jiangtao ZHANG.

Discussion

Danzig (1971) originally described *A. tesquorum* from Primor'ye Kray of Russia on *Cleistogenes sinensis,* and stated that the adult female of this species is much closer to *A. graminis*, but differs from the latter by *A. tesquorum* with 2 types of trilocular pores and tubular ducts whereas *A. graminis* with only 1 type of trilocular pores and tubular ducts. Tang (1992) found 2 types of trilocular pores and tubular ducts in some adult females of *A. graminis.* Therefore he considered those two species to likely be the same species. The first instar nymph of *A. tesquorum* is first described and illustrated here. After comparison with the first instar nymph of *A. graminis* described by Yang & Kosztarab (1967), it is found that the first instar nymphs of those two species are similar in: 1) clypeolabral shield without hornlike structures and with 2 setae; 2) anterior pair of dorsal ostioles absent, posterior pair between sixth and seventh abdominal segments, with 1 trilocular pore on each lip and 1 seta on anterior lip; 3) spiracles in 2 pairs, small trumpet-shaped, each spiracle associated with 2 trilocular pores; 4) circulus apparently absent; and 5) claw without denticle. However, the differences are mainly in: *A. tesquorum* having 3 types of trilocular pores, abdominal dorsum with 4 continuous longitudinal rows and 1 interrupted median row of trilocular pores, whereas *A. graminis* has one type of trilocular pore, abdominal dorsum with 6 continuous longitudinal rows and 1 interrupted median row of trilocular pores. So, based on morphology of first instar nymph, we considered *A. tesquorum* and *A. graminis* as two separate and distinct species.

The first-instar nymph of *Nesticoccus sinensis* was described by Xie and Yan (1983) and Tang (1992), but without much detail. Here we redescribe the first-instar nymph of *N. sinensis* in detail which is helpful in understanding the relationship between genus *Nesticoccus* and other genera in family Pseudococcidae.

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